

CLAIMS

1. A method of inducing an antigen specific immune response in a subject,  
comprising:

5 administering to the subject in order to induce an antigen specific immune response an antigen and a combination of adjuvants, wherein the combination of adjuvants includes at least one oligonucleotide containing at least one unmethylated CpG dinucleotide and at least one non-nucleic acid adjuvant, and wherein the combination of adjuvants is administered in an effective amount for inducing a synergistic adjuvant response.

10 2. The method of claim 1, wherein the non-nucleic acid adjuvant is an adjuvant that creates a depo effect.

15 3. The method of claim 2, wherein the adjuvant that creates a depo effect is selected from the group consisting of alum, emulsion-based formulations, mineral oil, non-mineral oil, water-in-oil emulsions, oil-in-water emulsions, Seppic ISA series of Montanide adjuvants, MF-59 and PROVAX.

20 4. The method of claim 1, wherein the non-nucleic acid adjuvant is an immune stimulating adjuvant.

25 5. The method of claim 4, wherein the immune stimulating adjuvant is selected from the group consisting of saponins, PCPP polymer, derivatives of lipopolysaccharides, MPL, MDP, t-MDP, OM-174 and *Leishmania* elongation factor.

6. The method of claim 1, wherein the non-nucleic acid adjuvant is an adjuvant that creates a depo effect and stimulates the immune system.

30 7. The method of claim 7, wherein the adjuvant that creates a depo effect and stimulates the immune system is selected from the group consisting of ISCOMS, SB-AS2, SB-AS4, non-ionic block copolymers, and SAF (Syntex Adjuvant Formulation).

8. The method of claim 1, wherein the combination of adjuvants is administered with a priming dose of antigen.

5 9. The method of claim 1, wherein the combination of adjuvants is administered with a boost dose of antigen.

10 10. The method of claim 8, wherein the subject is administered a boost dose of antigen and oligonucleotide containing at least one unmethylated CpG dinucleotide after the priming dose.

11. The method of claim 9, wherein the subject is administered a priming dose of antigen and oligonucleotide containing at least one unmethylated CpG dinucleotide before the boost dose.

15 12. The method of claim 1, wherein the oligonucleotide containing at least one unmethylated CpG dinucleotide has a sequence including at least the following formula:



wherein C and G are unmethylated, wherein  $X_1X_2$  and  $X_3X_4$  are nucleotides.

20 13. The method of claim 12, wherein the  $5' X_1 X_2 CGX_3 X_4 3'$  sequence is a non-palindromic sequence.

25 14. The method of claim 12, wherein the CpG-containing oligonucleotide is contained within a plasmid or viral vector.

15. The method of claim 12, wherein at least one nucleotide has a phosphate backbone modification.

30 16. The method of claim 15, wherein the oligonucleotide has 8 to 100 nucleotides.

17. The method of claim 15, wherein the phosphate backbone modification is a phosphorothioate or phosphorodithioate modification.

18. The method of claim 15, wherein the phosphate backbone modification occurs at  
5 the 5' end of the oligonucleotide.

19. The method of claim 15, wherein the phosphate backbone modification occurs at  
the 3' end of the oligonucleotide.

10 20. The method of claim 12, wherein  $X_1X_2$  are nucleotides selected from the group  
consisting of: GpT, GpG, GpA, ApA, ApT, ApG, CpT, CpA, CpG, TpA, TpT, and TpG; and  
 $X_3X_4$  are nucleotides selected from the group consisting of: TpT, CpT, ApT, TpG, ApG,  
CpG, TpC, ApC, CpC, TpA, ApA, and CpA.

15 21. The method of claim 12, wherein  $X_1X_2$  are selected from the group consisting of  
GpA and GpT and  $X_3X_4$  are TpT.

22. The method of claim 12, wherein  $X_1X_2$  are both purines and  $X_3X_4$  are both  
pyrimidines.

20 23. The method of claim 12, wherein  $X_2$  is a T and  $X_3$  is a pyrimidine.

24. The method of claim 12, wherein the oligonucleotide is 8 to 40 nucleotides in  
length.

25 25. The method of claim 12, wherein the oligonucleotide is isolated.

26. The method of claim 12, wherein the oligonucleotide is a synthetic  
oligonucleotide.

30 27. The method of claim 1, wherein the subject is an infant.

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28. The method of claim 1, wherein the antigen is derived from an infectious organism selected from the group consisting of a virus, bacterium, fungus and parasite.

5 29. The method of claim 1, wherein the antigen is a tumor antigen.

30. The method of claim 1, wherein the antigen is an allergen.

31. The method of claim 1, wherein the antigen is in the form of a crude extract.

10 32. The method of claim 1, wherein the antigen is in the form of a purified molecule including a protein or a polysaccharide.

15 33. The method of claim 1, wherein the antigen is in the form of a recombinant molecule including a protein, polypeptide, peptide or peptide mimic of a polysaccharide antigen.

20 34. The method of claim 1, wherein the non-nucleic acid adjuvant by itself give a Th2 immune response (e.g. alum) but when used in combination with the CpG oligonucleotide gives a Th1 response.

25 35. The method of claim 1 wherein the non-nucleic acid adjuvant by itself gives a Th1 immune response (e.g., MPL) but when used in combination with the CpG oligonucleotide gives a stronger Th1 response.

30 36. A composition of a synergistic combination of adjuvants, comprising:  
an effective amount for inducing a synergistic adjuvant response of a combination of adjuvants, wherein the combination of adjuvants includes at least one oligonucleotide containing at least one unmethylated CpG dinucleotide and at least one non-nucleic acid adjuvant.

37. The composition of claim 36, wherein the non-nucleic acid adjuvant is an adjuvant that creates a depo effect.

38. The composition of claim 37, wherein the adjuvant that creates a depo effect is selected from the group consisting of alum, emulsion based formulations, mineral oil, non-mineral oil, water-in-oil emulsions, water-in-oil-in-water emulsions, Seppic ISA series of Montanide adjuvants; MF-59; and PROVAX.

39. The composition of claim 36, wherein the non-nucleic acid adjuvant is an immune stimulating adjuvant.

40. The composition of claim 39, wherein the immune stimulating adjuvant is selected from the group consisting of saponins, PCPP polymer; derivatives of lipopolysaccharides, MPL, MDP, t-MDP, OM-174 and *Leishmania* elongation factor.

41. The composition of claim 36, wherein the non-nucleic acid adjuvant is an adjuvant that creates a depo effect and stimulates the immune system.

42. The composition of claim 41, wherein the adjuvant that creates a depo effect and stimulates the immune system is selected from the group consisting of ISCOMS, SB-AS2, AS2, SB-AS4, non-ionic block copolymers and SAF.

43. The composition of claim 36, wherein the composition also includes an antigen that is selected from the group consisting of peptides, polypeptides, cells, cell extracts, polysaccharides, polysaccharide conjugates, lipids, glycolipids, carbohydrates, viruses, viral extracts and antigens encoded within nucleic acids.

44. The composition of claim 43, wherein the antigen is derived from an infectious agent selected from the group consisting of a virus, bacterium, fungus and parasite.

45. The composition of claim 43, wherein the antigen is a tumor antigen.

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46. The composition of claim 43, wherein the antigen is an allergen.

47. A method for immunizing an infant, comprising:

5 administering to an infant an antigen and an oligonucleotide containing at least one unmethylated CpG dinucleotide and at least one non-nucleic acid adjuvant in an effective amount for inducing cell mediated immunity in the infant.

10 48. The method of claim 47, further comprising administering at least one non-nucleic acid adjuvant.

49. The method of claim 48, wherein the non-nucleic acid adjuvant is an adjuvant that creates a depo effect.

15 50. The composition of claim 49, wherein the adjuvant that creates a depo effect is selected from the group consisting of alum, emulsion based formulations, mineral oil, non-mineral oil, water-in-oil emulsions, water-in-oil-in-water emulsions, Seppic ISA series of Montanide adjuvants; MF-59; and PROVAX.

20 51. The method of claim 48, wherein the non-nucleic acid adjuvant is an immune stimulating adjuvant.

25 52. The composition of claim 51, wherein the immune stimulating adjuvant is selected from the group consisting of saponins, PCPP polymer; derivatives of lipopolysaccharides, MPL, MDP, t-MDP, OM-174 and *Leishmania* elongation factor.

53. The method of claim 47, wherein the non-nucleic acid adjuvant is an adjuvant that creates a depo effect and stimulates the immune system.

30 54. The composition of claim 53, wherein the adjuvant that creates a depo effect and stimulates the immune system is selected from the group consisting of ISCOMS, SB-AS2,

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AS2, SB-AS4, non-ionic block copolymers and SAF.

55. The method of claim 47, wherein the oligonucleotide is administered with a priming dose of antigen.

56. The method of claim 48, wherein the non-nucleic acid adjuvant is administered with a boost dose of antigen.

57. The method of claim 55, wherein the subject is administered a boost dose of antigen and oligonucleotide containing at least one unmethylated CpG dinucleotide after the priming dose.

58. The method of claim 56, wherein the subject is administered a priming dose of antigen and oligonucleotide containing at least one unmethylated CpG dinucleotide before the boost dose.

59. The method of claim 48, wherein the subject is administered a priming dose of antigen and oligonucleotide containing at least one unmethylated CpG dinucleotide and a boost dose of antigen, oligonucleotide and non-nucleic acid adjuvant.

60. The method of claim 47, wherein the oligonucleotide containing at least one unmethylated CpG dinucleotide has a sequence including at least the following formula:



wherein C and G are unmethylated, wherein  $X_1 X_2$  and  $X_3 X_4$  are nucleotides.

61. The method of claim 60 wherein the CpG-containing oligonucleotide is contained within a plasmid or viral vector.

62. The method of claim 60, wherein the  $5' X_1 X_2 C G X_3 X_4 3'$  sequence is a non-palindromic sequence.

63. The method of claim 60, wherein at least one nucleotide has a phosphate backbone modification

64. The method of claim 63, wherein the oligonucleotide has 8 to 100 nucleotides.

65. The method of claim 63, wherein the phosphate backbone modification is a phosphorothioate or phosphorodithioate modification.

66. The method of claim 63, wherein the phosphate backbone modification occurs at the 5' end of the oligonucleotide.

67. The method of claim 63, wherein the phosphate backbone modification occurs at the 3' end of the oligonucleotide.

68. The method of claim 60, wherein  $X_1X_2$  are nucleotides selected from the group consisting of: GpT, GpG, GpA, ApA, ApT, ApG, CpT, CpA, CpG, TpA, TpT, and TpG; and  $X_3X_4$  are nucleotides selected from the group consisting of: TpT, CpT, ApT, TpG, ApG, CpG, TpC, ApC, CpC, TpA, ApA, and CpA.

69. The method of claim 60, wherein  $X_1X_2$  are selected from the group consisting of GpA and GpT and  $X_3X_4$  are TpT.

70. The method of claim 60, wherein  $X_1X_2$  are both purines and  $X_3X_4$  are both pyrimidines.

71. The method of claim 60, wherein  $X_2$  is a T and  $X_3$  is a pyrimidine.

72. The method of claim 60, wherein the oligonucleotide is 8 to 40 nucleotides in length.

73. The method of claim 59, wherein the oligonucleotide is isolated.



74. The method of claim 59, wherein the oligonucleotide is a synthetic oligonucleotide.

75. A method of inducing a Th1-type immune response in a subject, comprising:  
administering to the subject in order to induce a Th1 immune response a combination of adjuvants, wherein the combination of adjuvants includes at least one oligonucleotide containing at least one unmethylated CpG dinucleotide and at least one non-nucleic acid adjuvant, and wherein the combination of adjuvants is administered in an effective amount for inducing a Th1-type immune response.

76. The method of claim 75, wherein the non-nucleic acid adjuvant is an adjuvant that creates a depo effect.

77. The composition of claim 76, wherein the adjuvant that creates a depo effect is selected from the group consisting of alum, emulsion based formulations, mineral oil, non-mineral oil, water-in-oil emulsions, water-in-oil-in-water emulsions, Seppic ISA series of Montanide adjuvants; MF-59; and PROVAX.

78. The method of claim 75, wherein the non-nucleic acid adjuvant is an immune stimulating adjuvant.

79. The composition of claim 78, wherein the immune stimulating adjuvant is selected from the group consisting of saponins, PCPP polymer; derivatives of lipopolysaccharides, MPL, MDP, t-MDP, OM-174 and *Leishmania* elongation factor.

80. The method of claim 75, wherein the non-nucleic acid adjuvant is an adjuvant that creates a depo effect and stimulates the immune system.

81. The method of claim 78, wherein the adjuvant that creates a depo effect and stimulates the immune system is selected from the group consisting of ISCOMS; SB-AS2; SB-AS4; non-ionic block copolymers and SAF.

82. The method of claim 75, wherein the combination of adjuvants is administered simultaneously.

83. The method of claim 75, wherein the combination of adjuvants is administered sequentially.

84. The method of claim 75, wherein the combination of adjuvants is administered in an effective amount for inducing a synergistic Th1 immune response.

85. The method of claim 75, wherein the oligonucleotide containing at least one unmethylated CpG dinucleotide has a sequence including at least the following formula:



wherein C and G are unmethylated, wherein  $X_1 X_2$  and  $X_3 X_4$  are nucleotides.

86. The method of claim 85, wherein the  $5' X_1 X_2 CGX_3 X_4 3'$  sequence is a non-palindromic sequence.

87. The method of claim 85, wherein at least one nucleotide has a phosphate backbone modification.

88. The method of claim 87, wherein the oligonucleotide has 8 to 100 nucleotides.

89. The method of claim 87, wherein the phosphate backbone modification is a phosphorothioate or phosphorodithioate modification.

90. The method of claim 87, wherein the phosphate backbone modification occurs at the 5' end of the oligonucleotide.

91. The method of claim 87, wherein the phosphate backbone modification occurs at the 3' end of the oligonucleotide.

92. The method of claim 85, wherein  $X_1X_2$  are nucleotides selected from the group consisting of: GpT, GpG, GpA, ApA, ApT, ApG, CpT, CpA, CpG, TpA, TpT, and TpG; and  $X_3X_4$  are nucleotides selected from the group consisting of: TpT, CpT, ApT, TpG, ApG, CpG, TpC, ApC, CpC, TpA, ApA, and CpA.

93. The method of claim 85, wherein  $X_1X_2$  are selected from the group consisting of GpA and GpT and  $X_3X_4$  are TpT.

94. The method of claim 85, wherein  $X_1X_2$  are both purines and  $X_3X_4$  are both pyrimidines.

95. The method of claim 85, wherein  $X_2$  is a T and  $X_3$  is a pyrimidine.

96. The method of claim 85, wherein the oligonucleotide is 8 to 40 nucleotides in length.

97. The method of claim 85, wherein the oligonucleotide is isolated.

98. The method of claim 85, wherein the oligonucleotide is a synthetic oligonucleotide.